

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K042542

B. Purpose for Submission:

New Device

C. Measurand:

Analysis of CEP XY probes in opposite-sex bone marrow transplant recipient specimens

D. Type of Test:

Computer-assisted image analyzer for fluorescence in situ hybridization (FISH)

E. Applicant:

Applied Imaging Corporation

F. Proprietary and Established Names:

Applied Imaging CytoVision™ CEP XY

G. Regulatory Information:

1. Regulation section:

21 CFR 866.4700, Automated Fluorescent in situ Hybridization (FISH) Enumeration Systems (Applied Imaging CytoVision™ system)

No regulation for CEP XY probes

2. Classification:

II (Applied Imaging CytoVision™ system)

Unclassified (CEP XY probes)

3. Product code:

NTH, System, automated scanning microscope and image analysis, for fluorescence in situ hybridization (FISH) assays (Applied Imaging CytoVision™ system).

MAO, DNA-Probe, human chromosome (CEP XY probes)

4. Panel:

Immunology 82 (Applied Imaging CytoVision™ system)

Hematology 81 (CEP XY probes)

H. Intended Use:

1. Intended use(s):

The Applied Imaging CytoVision™ system is an automated scanning microscope and image analysis system. It is intended for *in vitro* diagnostic use as an aid in chromosomal analysis. CytoVision assists in the location of interphase and metaphase nuclei on standard microscope slides using both brightfield and fluorescent microscopy.

This particular CytoVision software application is an accessory to the CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit (Vysis, Inc. Downer's Grove, IL) and is limited to the analysis of CEP XY probes via high magnification capture and analysis of interphase nuclei. CEP XY is indicated for use to assess the effectiveness of bone marrow transplantation in opposite-sex transplants.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

The Applied Imaging CytoVision™ system

I. Device Description:

The CytoVision CEP XY system is an integrated system combining the scanning, re-location, capture and analysis functions of CytoVision with a FISH signal analysis database and Review program. The system has features for finding cells or metaphases on brightfield or fluorescent slides. The software package allows the user to **Scan, Review, Capture** and **Analysis** of their images.

The CytoVision™ CEP XY system consists of an Intel-based computer (PC) with Windows Operating system, DVD-RAM, Monitor, keyboard, mouse, Graphic card, Motorized Microscope with brightfield and fluorescence capability and proper objectives, Set of dichroic filters, Coolsnap Camera, Frame Grabber, Motorized stage with IMS controller and joystick, and Additional third party software (Crystal Reports, SQL Server, Norton Anti-Virus, Executive Software Diskeeper). The Intel-based PC operates the instrument through the software program that coordinates control of the automated microscope. The automated microscope includes motorized filter turret containing fluorescence filters, focus control, brightfield and fluorescence lamps, objective nosepiece and condenser. These components can be controlled through the software. The Monochrome CCD camera is used to acquire the microscope images, which are then transferred to the PC through the image acquisition board for subsequent analysis by the software algorithm.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Cytoscan

2. Predicate 510(k) number(s):

K864955

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for use	The Applied Imaging CytoVision™ is an automated scanning microscope and image analysis system. It is intended for <i>in vitro</i> diagnostic use as an aid in chromosomal analysis.	Same
Method of cell detection	Colorimetric pattern recognition by microscopic examination of prepared cells by size, shape, hue, and intensity as observed by an automated computer controlled microscope and/or by visual observation by a health care professional.	Same
Device components	<ul style="list-style-type: none">Automated microscope	Same

Similarities		
Item	Device	Predicate
	<ul style="list-style-type: none"> • Video camera • PC with windows-based operating system • Keyboard and control panel • Color monitor for display of information • Color printer for reports • Dual Control Stick 	
Light source	Halogen lamp	Same

Differences		
Item	Device	Predicate
Indications for use	CytoVision assists in the location of interphase and metaphase nuclei on standard microscope slides using both brightfield and fluorescent microscopy.	Cytoscan assists in the location of metaphase nuclei on standard microscope slides using both brightfield and fluorescent microscopy.
Clinical application	CytoVision™ system is intended for in vitro diagnostic use as an aid in chromosomal analysis. It improves the user's ability to identify particular cells; for this application, measuring XX and XY chromosome percentages in gender mismatched bone marrow transplant recipient samples.	Cytoscan is designed to assist cytogeneticists and technicians in locating suitable chromosomes for evaluation. It is a metaphase finder and computer aided chromosome analysis system. The machine does not diagnose; it merely locates and displays chromosomes.
Microscope objectives	20x, 40x, 60x, 100x	10x, 20x, 40x
Software Application	Analyze CEP XY probes via high magnification capture and analysis of interphase and metaphase nuclei.	The computer program locates only metaphase spreads.

K. Standard/Guidance Document Referenced (if applicable):

None provided.

L. Test Principle:

CytoVision improves the user's ability to identify particular cells; for this application, measuring XX and XY chromosome percentages in gender mismatched bone marrow transplant recipient samples. The device reports the counts of XX and XY cells observed in a sample of approximately 200 cells from a bone-marrow aspirated sample, and the results are intended for use by physicians in evaluating engraftment efficiency by the marrow-transplant recipient. Currently, these samples are generally read by manual microscopic

examination of the same approximate 200-cell count. The cell counts containing XX and XY chromosome pairs are then converted to percentages for interpretation by the physician.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Three separate precision studies were conducted to examine the various CytoVision™ CEP XY components of variation reported as XX% and XY%. Study 1 consisted of three separate runs on five evaluation samples (2 control samples and 3 patient samples) on a single CytoVision™ instrument on a single day. Study 2 consisted of three runs, each on a different day, on a single instrument. There was a minimum of two days between the CytoVision™ runs. Study 3 consisted of a single run on three different instruments. For each study, each run involved duplicate slides of each of the study materials (10 slides/study). Slides were stained with the Vysis CEP XY DNA probe kit, and each study was designed to estimate different components of variance in total imprecision.

Tables 1, 2, and 3 tabulate the results of the three precision experiments. Variance component estimates are provided in each table for both the larger and smaller proportions. All total error standard deviations were at or below 1%, indicating highly consistent results across instruments, days, and runs. Percent coefficients of variation (%CVs) for the larger portions were at or below 1%, and %CVs for the smaller portion ranged between 1% and 23%. The larger %CVs for the smaller proportions are due to the effect of dividing the standard deviation by a smaller number, as compared to dividing the standard deviation by a larger number.

Table 1
Precision Study 1
Within-day Within-Instrument
CytoVision™ XX% and XY%

Sample	Mean	Within-run Var.	Between-run Var.	Total Var.	Total SD	Within-run CV%	Between-run CV%	Total CV%
Control 1 XX	95.55%	0.358%	0.014%	0.372%	0.610%	0.626	0.122	0.64
Control 1 XY	4.45%	0.358%	0.014%	0.372%	0.610%	13.434	2.627	13.69
Control 2 XX	4.93%	0.096%	0.006%	0.102%	0.320%	6.300	1.568	6.49
Control 2 XY	95.07%	0.096%	0.006%	0.102%	0.320%	0.327	0.081	0.34
Patient A XX	4.53%	0.106%	0.000%	0.106%	0.325%	7.178	0.000	7.18
Patient A XY	95.47%	0.106%	0.000%	0.106%	0.325%	0.340	0.000	0.34
Patient B XX	25.50%	0.104%	0.000%	0.104%	0.322%	1.264	0.000	1.26
Patient B XY	74.50%	0.104%	0.000%	0.104%	0.322%	0.433	0.000	0.43
Patient C XX	76.58%	0.079%	0.000%	0.153%	0.391%	0.368	0.000	0.37
Patient C XY	23.42%	0.079%	0.000%	0.153%	0.391%	1.204	0.000	1.20

Var. = variance SD = standard deviation CV = %CV

Table 2
Precision Study 2
Between-day Within-Instrument
CytoVision™ XX% and XY%

Sample	Mean	Within-run Var.	Between-run Var.	Total Var.	Total SD	Within-run CV%	Between-run CV%	Total CV %
Control 1 XX	95.45%	0.124%	0.490%	0.614%	0.784%	0.369	0.733	0.82
Control 1 XY	4.55%	0.124%	0.490%	0.614%	0.784%	7.753	15.394	17.24
Control 2 XX	4.80%	0.104%	0.000%	0.104%	0.322%	6.708	0.000	6.71
Control 2 XY	95.20%	0.104%	0.000%	0.104%	0.322%	0.338	0.000	0.34
Patient A XX	4.56%	0.064%	0.001%	0.065%	0.255%	5.538	0.785	5.59
Patient A XY	95.44%	0.064%	0.001%	0.065%	0.255%	0.264	0.037	0.27
Patient B XX	25.45%	0.119%	0.000%	0.119%	0.345%	1.355	0.000	1.36
Patient B XY	74.55%	0.119%	0.000%	0.119%	0.345%	0.463	0.000	0.46
Patient C XX	76.68%	0.026%	0.003%	0.029%	0.170%	0.211	0.068	0.22
Patient C XY	23.32%	0.026%	0.003%	0.029%	0.170%	0.695	0.223	0.73

Var. = variance SD = standard deviation CV = %CV

Table 3
Precision Study 3
Within-day Between-Instruments
CytoVision™ XX% and XY%

Sample	Mean	Within-run Var.	Between-run Var.	Total Var.	Total SD	Within-run CV%	Between-run CV%	Total CV%
Control 1 XX	95.09%	0.494%	0.000%	0.494%	0.703%	0.739	0.000	0.74
Control 1 XY	4.91%	0.494%	0.000%	0.494%	0.703%	14.317	0.000	14.32
Control 2 XX	5.57%	1.031%	0.751%	1.782%	1.335%	18.234	15.558	23.97
Control 2 XY	94.43%	1.031%	0.751%	1.782%	1.335%	1.076	0.918	1.41
Patient A XX	4.71%	0.088%	0.000%	0.088%	0.297%	6.298	0.000	6.30
Patient A XY	95.29%	0.088%	0.000%	0.088%	0.297%	0.311	0.000	0.31
Patient B XX	25.46%	0.362%	0.000%	0.362%	0.602%	2.365	0.000	2.36
Patient B XY	74.54%	0.362%	0.000%	0.362%	0.602%	0.808	0.000	0.81
Patient C XX	76.63%	0.435%	0.001%	0.435%	0.660%	0.860	0.039	0.86
Patient C XY	23.37%	0.435%	0.001%	0.435%	0.660%	2.821	0.128	2.82

Var. = variance SD = standard deviation CV = %CV

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The analytical traceability of the system depends on the Vysis CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit.

ProbeChek slides, supplied by Vysis, are designed for use as controls for interphase FISH and laboratory quality control, specifically, for the CEP X/Y IVD Kit.

ProbeChek Low Level Male Control Slides (95% XX, 5% XY) and ProbeChek Low Level Female Control Slides (95% XY, 5% XX) are tested and optimized for use in Fluorescence *in situ* Hybridization experiments. These slides are manufactured from a mixture of cultured normal male and female lymphoblast cell lines.

d. Detection limit:

Not Applicable.

e. Analytical specificity:

The analytical specificity of the test result is dependent on the analytical performance of the Vysis CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit.

f. Assay cut-off:

The assay cut-off of the test result is dependent on the analytical performance of the Vysis CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit. The pathologist must follow the recommendations of the CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit.

2. Comparison studies:

a. Method comparison with predicate device:

For method comparison, three different clinical study sites each supplied specimens from 20 different patients who were at differing stages of the post-transplant follow-up process. The range of post-transplant time-spans was between 30 days and 8+ years. The slides were stained with the Vysis CEP XY probe kit, and the respective manual readings were always submitted.

The data were plotted on a scatter plot showing the comparative results of the manual counts and the CytoVision™ results. Figure 1 shows this comparison for the XX% chromosome percentages, along with the ordinary least squares regression model fit and the exhibited correlation coefficient. The estimated regression slope was 1.0003, with an intercept of -0.2013 and a correlation coefficient of 0.9991. Figure 2 shows the same relationship for the XY% results and Figure 3 shows the combination (XX%/XY%) results.

Figure 1 Concordance Comparison CytoVision™ XX% vs Manual XX%

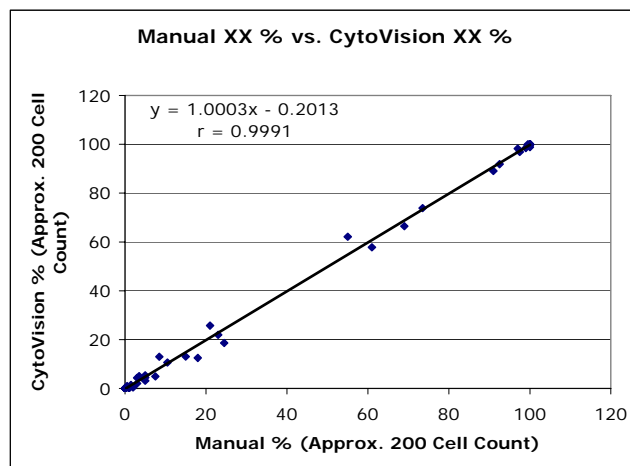


Figure 2 Concordance Comparison CytoVision™ XY% vs Manual XY%

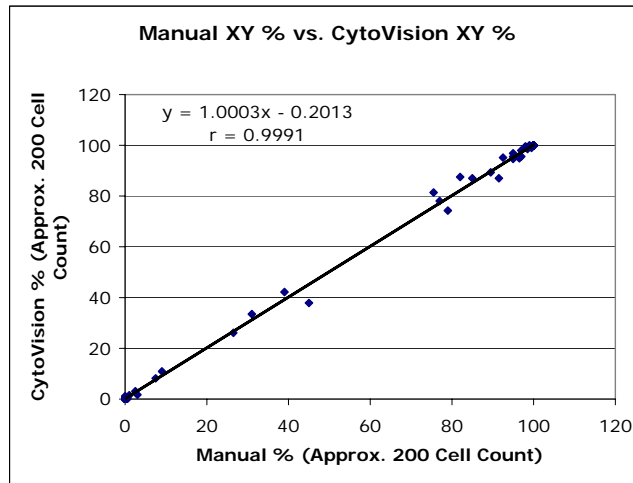
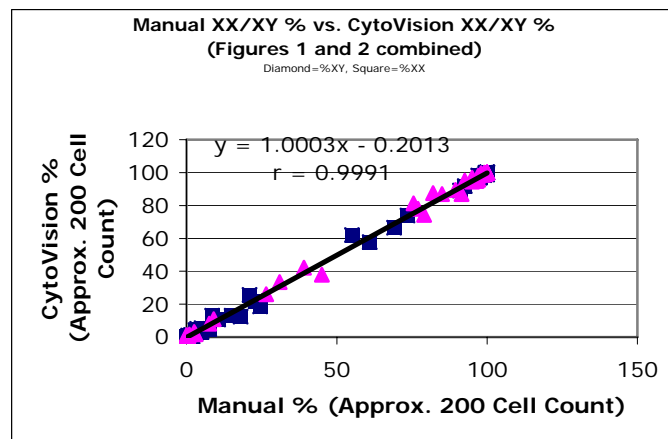


Figure 3 Concordance Comparison CytoVision™ XX%/XY% vs Manual XX%/XY%



- b. *Matrix comparison:*
Not applicable.
3. Clinical studies:
 - a. *Clinical Sensitivity:*
The clinical sensitivity of the test result is dependent on the analytical performance of the Vysis CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit.
The pathologist must follow the recommendations of the CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit.
 - b. *Clinical specificity:*
The clinical specificity of the test result is dependent on the analytical performance of the Vysis CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit.
The pathologist must follow the recommendations of the CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit.
 - c. *Other clinical supportive data (when a. and b. are not applicable):*
Not Applicable.

4. Clinical cut-off:

The clinical cut-off of the test result is dependent on the analytical performance of the Vysis CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit. The pathologist must follow the recommendations of the CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit.

5. Expected values/Reference range:

Not Applicable.

N. Instrument Name:

Applied Imaging CytoVision™

O. System Descriptions:

1. Modes of Operation:

Semi-automated computer assisted interpretation.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ☒ or No ☐

3. Specimen Identification:

Slides are created in an open case and cells are created in a slide. Slides and cells are numbered automatically but can be renamed after they are created. Each case is a separate directory on the hard drive. Slides and cells are subdirectories contained within the cases and are used to organize the images. The slides and cells are created as needed using the navigator. There is no limit on the number of slides and cells in a case. They can be renamed or deleted but cannot be moved between cases. Case details stores information about the patient or case. It has commonly used fields such as patient name and date of birth, and an area for creating custom field titles.

4. Specimen Sampling and Handling:

The CytoVision system does not have a slide loader or an automated bar code reader. The slides are manually numbered, manually loaded on the stage and images are captured automatically. The Cytovision™ CEP XY system software package allows the user to scan, review, capture and analyze their images on the monitor screen. In the **Scan** feature, the user uses the "Scan Wizard" and automatically scans (typically with a 10x objective) a predefined area to find cells (metaphase or interphase) of interest. This creates a list of objects for Review, referred to as the "metlist". Using the **Review** feature, the user find objects and delete objects which are not of interest (e.g. image artifacts). This will update the metlist. In the **Capture** feature, the user uses the "Capture Wizard" and captures all objects in the metlist with a higher magnification (typically a 60x objective). Using the **Analysis** function, the user reviews the scoring created by the system and make the necessary corrections.

5. Assay Types:

Computer-assisted image analysis of fluorescence in situ hybridization signals in interphase and metaphase nuclei of cells in gender mismatched bone marrow transplant recipient samples.

6. Reaction Types:

Fluorescent microscopy

7. Calibration:

The calibration of the Cytovision™ system is split into two sections: (a) SPOT

calibration, and (b) Pre-scan calibration. Pre-scan calibration should only need to be done at initial installation or if the stage has been removed and re-installed. SPOT calibration defines the stage and microscopes automatic components, including the filter and objective positions, calculates spatial calibration of stage steps per pixel and microns per pixel for each objective lens.

6. Quality Control:

The accuracy of the system depends on the laboratory following the quality control instructions recommended in the labeling of the fluorescence in situ hybridization (FISH) assay kit associated with the Cytovision™.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

Refer to Section M.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.